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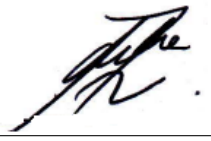
Signature	:	
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CONTINUOUS GLUCOSE MONITORING (CGM) USING LAB ON CHIP (LOC)

NURUL ATIRAH BINTI MUSTAFFA

A final year project report submitted in fulfilment of the
requirements for the award of the degree of
Bachelor of Electrical Engineering (Electric-Electronic)

Faculty of Electrical Engineering
Universiti Teknologi Malaysia

JUNE 2015

I declare that this final year project report entitled *"CONTINUOUS GLUCOSE MONITORING (CGM) USING LAB ON CHIP (LOC)"* is the result of my own research except as cited in the references. The final year project report has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special thanks to

My beloved parents and family that always keep their prayers for me

My supervisor that guide me through hardship

My friends who always assists and give me moral support

And also to

Everyone that help me through this research

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ABSTRACT

Glucose is an important parameter that has to be considered before undergoing any surgery. Variation of glucose level in body will affect the decisions during surgery. Utilizing lab on chip (LOC), glucose measurement can be taken continuously during operation time, in order to give any sign if a problem occurs. LOC with channel of two inlets and one outlet can be implemented using polydimethyl siloxane (PDMS) that is designed using Solidworks program. A screen printed electrode (SPE) is placed in the middle of the channel inside the LOC to measure the amperometric reaction using Emstat between glucose and glucose oxidase that is pumped in through both inlets. Glucose buffer solution and glucose oxidase is prepared using combination of phosphate buffer saline (PBS) and deionized water. The system performance is measured using PSTrace program. The result shows that, the current detected by the SPE is proportional to the concentration of glucose.

ABSTRAK

Glukosa adalah parameter penting yang perlu dipertimbangkan sebelum menjalani sebarang pembedahan. Perubahan paras glukosa dalam badan akan memberi kesan kepada keputusan semasa pembedahan . Menggunakan makmal pada cip (LOC), pengukuran glukosa boleh diambil secara berterusan sepanjang waktu pembedahan, untuk memberi sebarang tanda jika terdapat masalah. LOC dengan dua saluran masuk dan satu salur keluar boleh dilaksanakan menggunakan polydimethyl siloksan (PDMS) yang direka menggunakan program Solidworks. Elektrod bercetak skrin (SPE) diletakkan di tengah-tengah saluran dalam LOC untuk mengukur reaksi amperometric menggunakan Emstat antara glukosa dan glukosa oksidase yang disuntik melalui kedua-dua salur masuk. Cairan penampan glukosa dan glukosa oksidase disediakan menggunakan kombinasi garam fosfat penampan (PBS) dan air ternyahion. Prestasi sistem diukur menggunakan program PSTrace. Hasil menunjukkan, arus yang dikesan oleh SPE adalah berkadar terus dengan kepekatan glukosa.

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LIST OF ABBREVIATIONS

CGM	-	Continuous Glucose Monitoring
LOC	-	Lab On Chip
SPE	-	Screen Printed Electrode
PDMS	-	Polydimethyl Siloxane
PBS	-	Phosphate Buffer Solution
3D	-	3 Dimensional
GOD	-	Glucose Oxidase
MPC	-	Methacryloyloxyethyl Phosphorylcholine
Ag	-	Silver
AgCl	-	Silver Chloride
Pt	-	Platinum
MEMS	-	Micromechanical System
WE	-	Working Electrode
CE	-	Counter Electrode
RE	-	Reference Electrode
DC	-	Direct Current
USB	-	Universal Serial Bus
FYP1	-	Final Year Project 1
FYP2	-	Final Year Project 2
mg	-	Miligram
dl	-	Deci liter

LIST OF SYMBOLS

μ	-	micro

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CHAPTER 1

INTRODUCTION

This chapter gives the explanation about the project background, problem statement, objectives, as well as project scope.

1.1 Introduction

Glucose is an important aspect in human body where, its level in the blood is controlled by the secretion of insulin in the negative feedback system. For a diabetic patient, the blood glucose concentrations usually fall outside the normal range of 70-180mg/dl. This happened because their body does not secrete insulin or maybe there is derangement in both insulin secretion and action [1]. Usually, in surgical procedures, before a patient undergo any surgery, glucose measurement must be taken several times per day in the perioperative environment [2]. The measurement must be taken a week before the operation so that the insulin regimen can be adjusted according to the condition of the patient [3]. This is an important aspect that has to be considered especially in diabetic patient because they face more challenges during surgery compare to the non-diabetic patients. Many of the challenges occur due to inabilities to maintain a balance between insulin and its counter regulatory hormones [4].

Nowadays, several sensors have been developed in order to measure the glucose concentration, continuously in the human body. With the continuous glucose monitoring (CGM), the glucose concentration in the human body can be measured continuously for a long period of time [5]. Compare to the intermittent measurement, such as finger prick [6], CGM provides more information about the glucose level, such as frequency, magnitude, direction, duration, and cause of fluctuation in glucose level [7]. This information is useful if applied during the surgery because it can continuously

measure the glucose level of patients and give a sign if there are any problems that happened during the surgery.

1.2 Problem Statement

Based on the application in the operation room, the intermittent measurement that has been applied to measure the glucose level is very inconvenient [8] because the measurement must be taken several times in order to get the pattern of the glucose level in the patient's blood [4]. The measurement is taken only before the surgery and it is not being monitored during the operation [9]. This may provide risk to the patient as there is no indication in fluctuation of glucose level during the operation.

1.3 Objectives

This project aims to:

i. Design a system that can measure the glucose level continuously

ii. To fabricate lab on chip using PDMS materials

iii. Measure the system performance by using PSTrace program

1.4 Research Scope

In order to achieve the objectives stated in 1.3 , several scopes have been outlined. The scope includes Solidworks 2013, as a platform to design 3D image that will be used to make mold of lab on a chip. While, Polydimethylsiloxane (PDMS) will be used as material to fabricate the lab on a chip. Moreover, the screen printed electrode (SPE) will be used as a detector and the glucose level will be measured using the Emstat. Lastly, the PSTrace program will be used to analyze the performance of the CGM system.

1.5 Thesis Outline

This thesis consists of six chapters that include the introduction, literature review, research methodology, result and discussion, project management, conclusion as well as future works.

The first chapter gives the brief explanation about the project background as well as the problem statement that are related to the project. Based on the problem statement, several objectives have been introduced that aim to solve the problem issues with the guideline from the research scope.

In second chapter, discussion about literature review will take place that give an overview about previous project that are related with the CGM system or lab on chip fabrication. This chapter help to give a clear picture about the working principles of CGM system, types of sensor that are usually used in CGM system, methods of moving fluids inside microfluidic devices, and pattern of microfluidic device that usually use in designing lab on chip.

In third chapter, a brief explanation about project work flow, block diagram, software implementation in designing mold of lab on chip, steps fabrication of lab on chip using PDMS materials, and hardware implementation for CGM system.

The fourth chapter deals with the functionality of the system that analyze the result obtained based on the hardware and software implementation as well as the reaction from different concentration of glucose and glucose oxidase inside the channel of lab on chip that detected by SPE. The result based on cyclic voltametry and amperometric detection graph also included in this chapter.

The fifth chapter gives a brief explanation about the project management. Where, in this chapter, project schedule and project costing has been included.

Lastly, chapter six gives conclusion about the result obtained and give a brief explanation about the limitation and recommendation for future works.

CHAPTER 2

LITERATURE REVIEW

This chapter gives a brief review about the previous or similar projects of CGM system. It provides a clear understanding about type of glucose sensors that is suitable to be used in CGM system, methods of moving fluids inside microfluidic devices, and pattern of microfluidic device that usually use in designing lab on a chip.

2.1 Continuous Glucose Monitoring

Continuous glucose monitoring (CGM) is a way to measure the glucose level throughout a long period of time. CGM provides all information about the magnitude, duration, direction and causes of fluctuation in the glucose level [7].

2.2 Glucose Detection

The glucose detection is based on an amperometric approach of enzymatic oxidation between glucose and glucose oxidase (GOD) such as shown in Equation 2.1 [10]:

$$Glucose \dot{+} O_2 \xrightarrow{GOD} Gluconolactane \dot{+} H_2O_2$$

$$H_2O_2 \longrightarrow \quad O_2 \dot{+} 2H^+ \dot{+} 2e^- \tag{2.1}$$

The enzyme was immobilized onto the surface of working electrode which react with glucose to produce electric current [11].

2.2.1 Screen Printed Electrode

A research by H. Xu, G. Li, J.Wu *et al.* [12] provide a screen printed electrode as glucose detector such in Figure 2.1 . The sensor use carbon and Ag/AgCl ink to form a working electrode (WE), counter electrode (CE) and reference electrode (RE). GOD was immobilized onto the carbon electrode of WE and CE. While, silver were printed onto the three electrodes as a conductive lead. The sensor provides better response current which gives linear relationship with the increment of glucose concentration.

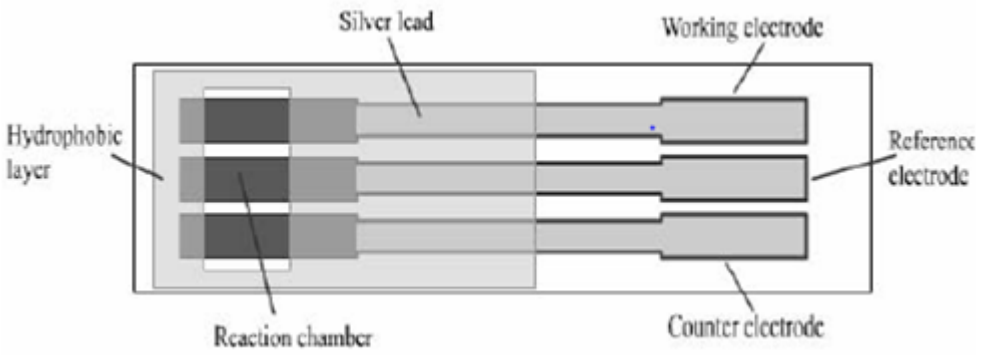

Figure 2.1: Screen Printed Electrode [12]

2.2.2 Glucose Sensor Based on Functional Polymers

H. Kudo, T. Sawada, E. Kazawa *et al.* [10], provide a glucose sensor using functional polymer such as polydimethylsiloxane (PDMS), and methacryloyloxyethyl phosphorylcholine (MPC) as its material with Pt and Ag/AgCl as its electrodes through a process called micromechanical system (MEMS). The sensor showed a better flexibility, such as shown in Figure 2.2 since it was fabricated using functional polymer. It also showed a good sensitivity towards different glucose concentration. However,

the performance decreases after rinsing the electrode with potassium ferricyanade as electron mediator. The decrease is due to the loss of electrons during rinsing.

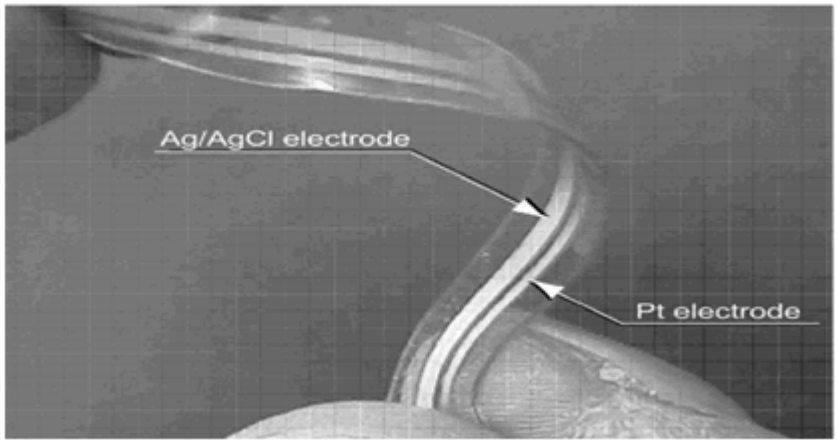

Figure 2.2: Glucose sensor based on functional polymer [10]

2.3 Lab on A Chip

Lab on chip is a microfluidic device that uses to integrate miniaturized laboratory function such as separation and analysis of mixture components on a single microprocessor chip using extremely small volume of fluids [13].

2.3.1 Methods of Moving Fluids inside Microfluidic Devices

The fluidic transport system is an important element that needs to be considered in microfluidic devices. B. Weigl, R. Bardell, C. Cabrera [14], have proposed several techniques for fluidic transport such as pressure driven flow, electroosmotic flow, electrokinetic flow control, and electrokinetic mixing. Thus, it concludes that electroosmotic flow provides very sensitive to the variations in both liquid decomposition and channel wall coating. It requires limited use of generic pumping solution while, pressure driven flow significantly less sensitive towards viscosity of fluids.

2.3.2 Pattern of Microfluidic Devices

Y. Atalay, P. Verboven, S. Vermeir *et a*l. in [15] provide model based design for microfluidic biochip that uses to detect multiple analytes. The design such in Figure 2.3 consists of five inlets and one outlet. Where, in this case, inlet 1 is used to inject a buffer solution, inlet 2 and 3 for sample volume, while, inlet 4 and 5 used to inject enzyme. Therefore, it stated that, the design was used for fast quantification of multiple components in the multiplexed microchannel reactor. Furthermore, since this model is designed to detect multiple analytes, thus, it is quite complex and not necessarily considered in detecting one sample of analytes such as glucose.

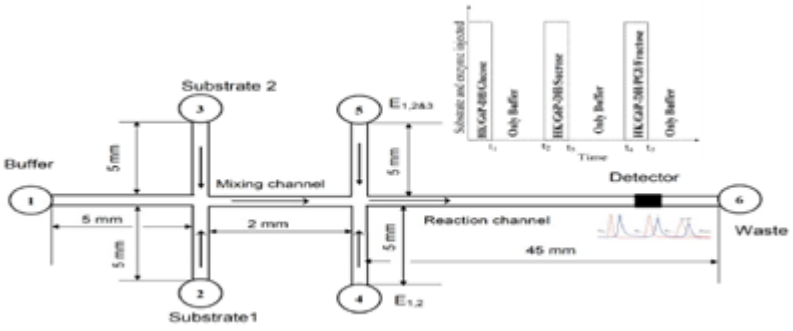

Figure 2.3: Model based design of microfluidic biochip [15]

2.3.3 Fabrication Process of Microfluidic Device

P. Abgrall, A. Gue, [16] show the fabrication of microfluidic device based on the PDMS casting process. As shown in Figure 2.4, the process begins by manufacturing the mould by bulk machining silicon frequently. Then, a mixture of silicon rubber and cross linking agent is cast on top of the silicon layer. After cross linking process, the elastomer is peeled off from the substrate. Then, access is created frequently with a part holder. After that, the part is transferred into another piece of PDMS. This technique gives property that makes the PDMS easy to close because it places the open structure on a flat substrate and easy to be stripped as PDMS is an elastomer. Furthermore, this technique is preferred to be done in a laboratory that did not have advanced technology tools as it uses material such as PDMS.

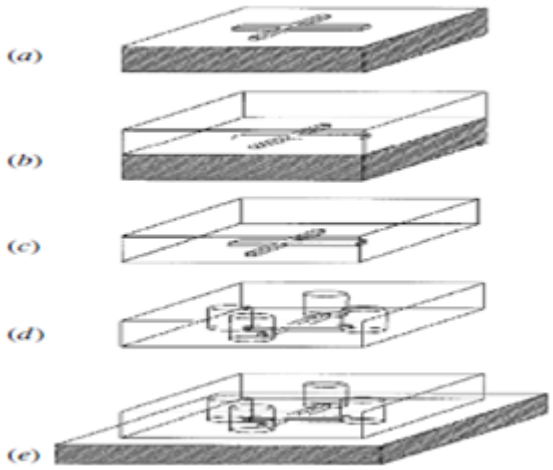

Figure 2.4: PDMS casting [16]

2.4 Conclusion

Based on the research that has been made for this project, the CGM system in this project will use screen printed electrode (SPE) as a detector because of its good sensitivity towards different glucose level. Furthermore, since this project only focus to detect and measure the glucose level, therefore, the pattern of lab on a chip will be designed by having two inlets for injection of glucose and GOD enzyme, together with one outlet to pump out the waste.

CHAPTER 3

RESEARCH METHODOLOGY

This chapter gives a brief explanation about project work flow, block diagram, experimental flow that involves in fabrication process of lab on chip using PDMS materials, preparation of glucose and glucose oxidase solution, as well as software and hardware implementation in designing CGM system. Besides that, this chapter also explain about the system specification.

3.1 Project Work Flow

This project is divided into three main stages which are designed lab on a chip that is suitable to be used in a CGM system using Solidworks 2013, fabrication process of lab on a chip using PDMS material, and lastly assembly of the hardware together and analyze the data using PSTrace.

3.2 Block Diagram

Figure 3.1 shows the block diagram for CGM system. A sample of glucose oxidase and glucose will be pumped into the inlet 1 and inlet 2 of lab on chip using peristaltic pump and motor driver that has been controlled by the Arduino Uno microcontroller. Three electrodes of potentiostat that is connected to the PSTrace programmed inside the computer will be connected to the three electrodes of SPE which is working electrode (WE), counter electrode (CE), and a reference electrode (RE) that has been integrated with the lab on a chip. The PSTrace program will analyze the output measured from the potentiostat.

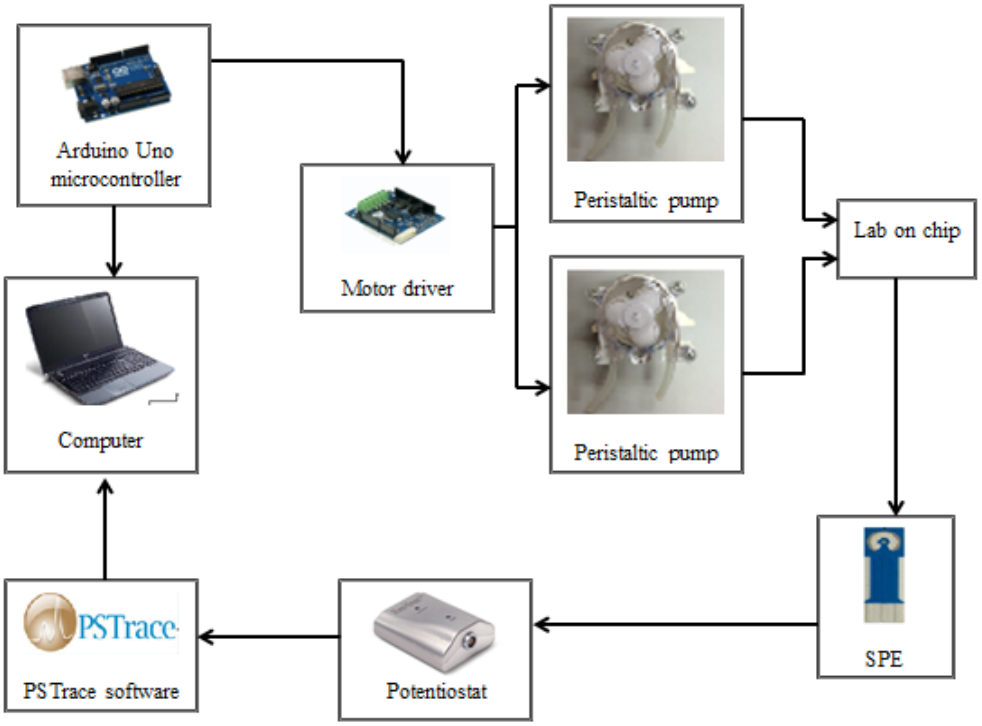

Figure 3.1: Block diagram of CGM process

3.2.1 Process Flow Chart

Figure 3.2 shows the process flow chart for the CGM system.

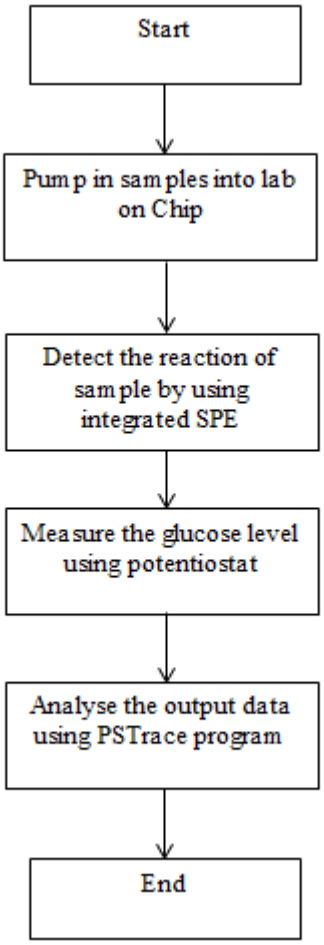
Start
Pump in samples into lab on Chip
Detect the reaction of sample by using integrated SPE
Measure the glucose level using potentiostat
Analyse the output data using PSTrace program
End

Figure 3.2: Process flow chart for the CGM System

3.3 Hardware Implementation

This project requires two peristaltic pumps, one motor driver, Arduino Uno microcontroller, a potentiostat and SPE as a detector for glucose. In order to control the speed of peristaltic pump, Arduino Uno microcontroller as well as the motor driver has been used. The Arduino Uno microcontroller board has been program using Arduino software. The program are then transferred into the board using USB cable. Additional 9V battery power supply has been used so that the Arduino Uno can standalone without the need to be connected to any machine. Figure 3.3 shows the hardware implementation to run the peristaltic pump.

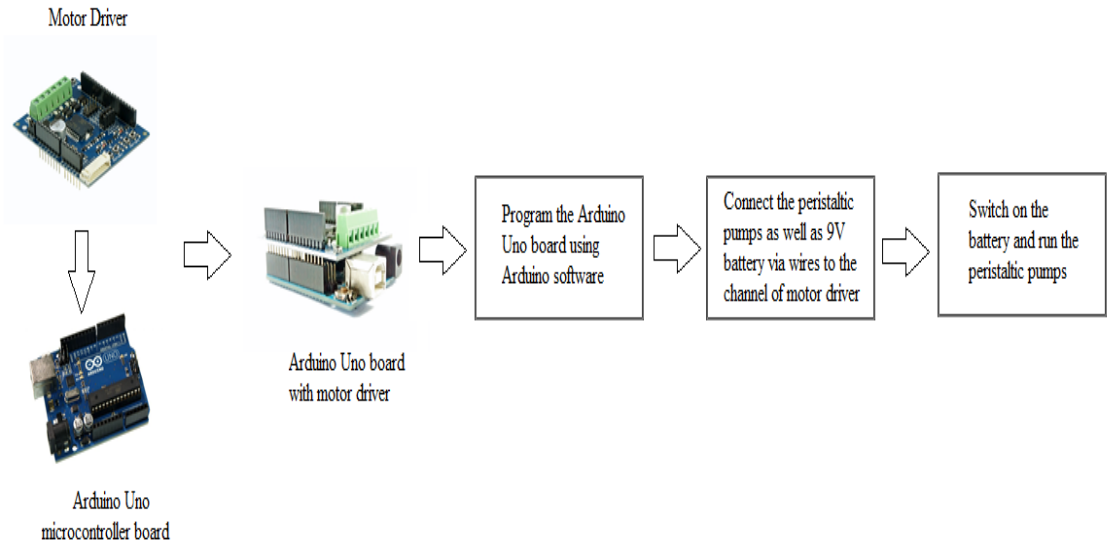
Motor Driver
Arduino Uno board with motor driver
Program the Arduino Uno board using Arduino software
Connect the peristaltic pumps as well as 9V battery via wires to the channel of motor driver
Switch on the battery and run the peristaltic pumps
Arduino Uno microcontroller board

Figure 3.3: Hardware implementation to run the peristaltic Pumps

3.3.1 Peristaltic Pump

This project uses 2 peristaltic pumps such as in Figure 3.4 in order to pump in sample volume of glucose oxidase and glucose solution into inlet 1 and inlet 2 of lab on a chip.

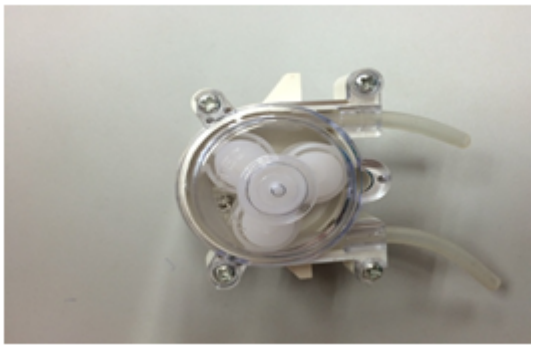

Figure 3.4: Peristaltic pump

Table 3.1 also shows the specification of the peristaltic pump. The peristaltic pumps have silicon tubing that can move the fluid from one place to another place safely. Furthermore, it also operate at motor voltage of 12V DC with 300 mA motor current which is quite small compared to other DC motor. The maximum flow rate that can be handle by this peristaltic pump is up to 100mL/min.

Table 3.1: Specification of peristaltic pump

Specifications	
Tube Material	Silicon
Working Temperature	$0^0C - 40^0C$
Motor Voltage	12V DC
Motor Current	300mA
Flow Rate	Up to 100mL/min
Weight	200g
Dimension	Diameter : 27mm, Length : 62mm

3.3.2 Motor Driver

This project requires one motor driver in order to control speed of the pump to pump in the sample volume into lab on chip. The motor driver used is the shield type that allows the motor driver to be stacked on top of Arduino Uno as in Figure 3.5. Furthermore, the motor driver used are compatible with arduino Uno and can be used to control two DC motor up to 2A for each channel. Since the peristaltic pump operate at 300mA, therefore, by using 2A motor driver it can be used to drive the peristaltic pumps.

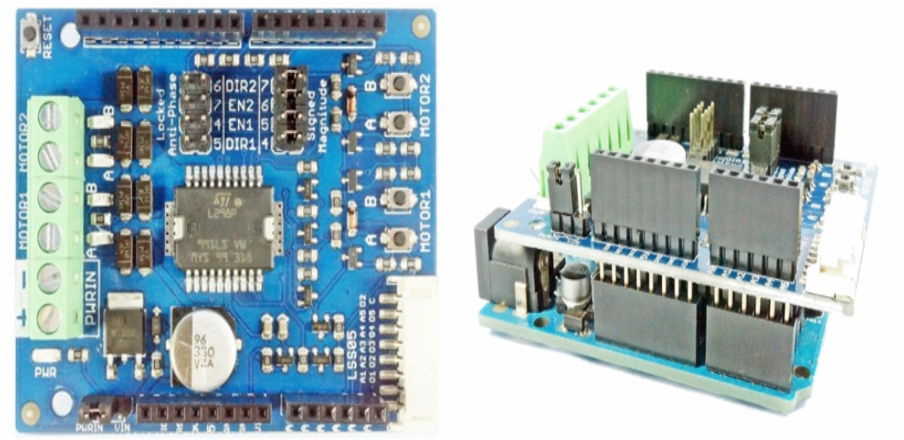

Figure 3.5: Motor driver

Table 3.2shows the specification and limitation of the motor driver. The motor driver can operate at maximum 2A current value and can be supplied with minimum 5V to 26V input power.

Table 3.2: Specification and limitation of motor driver

Parameter	Min	Typical	Max	Unit
Power Input Voltage (Motor supply voltage)	5	-	26	V
I_{MAX} (Maximum Continuous Motor Current)	-	-	2	A
I_{PEAK}(Peak Motor Current)	-	-	2	A
V_{IOH}(Logic Input-High Level)	3.3	-	5.5	V
V_{IOL}(Logic Input-Low Level)	0	0	0.5	V
Maximum PWM Frequency	-	-	10	KHz

3.3.3 Screen Printed Electrode (SPE)

Screen printed electrode (SPE) such as in Figure 3.6 contain three electrodes such as working electrode (WE), counter electrode (CE) and reference electrode (RE). It is used as a detector to detect the presence of glucose through the reaction of glucose and glucose oxidase inside Lab on a chip. In this project, screen printed electrode that are made up of gold material for its working and counter electrode as well as silver material for its reference electrode were used. This type of material were selected since its has more stability towards glucose reaction.

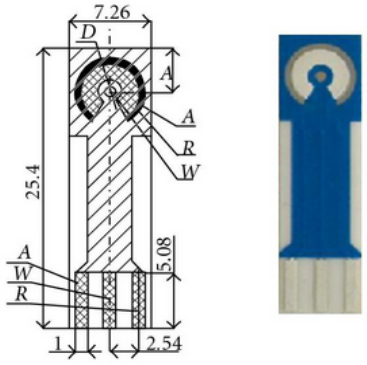

Figure 3.6: Screen printed electrode

3.3.4 Potentiostat

This project uses an Emstat potentiostat as in Figure 3.7 to measure the glucose level. It is connected to the working electrode (WE), counter electrode (CE) and reference electrode (RE) of SPE. The glucose level is a measure of the voltage difference between WE and RE as well as current flow between WE and CE. The Emstat is powered via USB and controlled by the PSTrace program that has been installed inside the computer. Emstat has eight current ranges that is from 1nA to 100mA with minimun resolution of 1pA.

Figure 3.7: Potentiostat

3.4 Experiment Flow

In this project, the experimental flow include the fabrication process of lab on chip using the PDMS material, preparation of solution for both glucose and glucose oxidase, as well as programming using Arduino Uno software to control the the speed of peristaltic pumps in order to pump in the solution samples into the LOC.

3.4.1 Fabrication of Lab on Chip (LOC)

In this process, before fabricating LOC using PDMS material, the Solidworks program were used to design the mould of LOC. The mold is designed in 50mm x 20mm dimension with 1mm diameter of Y and spiral shape channel. At the end of the spiral shape channel, 6mm circular diameter is designed that use to place the integrated SPE. Figure 3.8 shows the mold design of LOC using Solidworks.

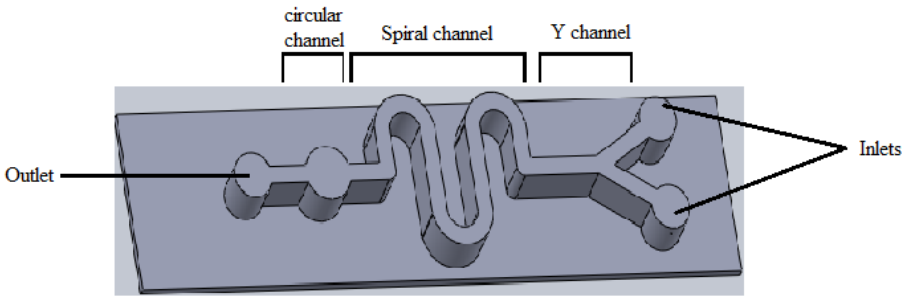

Figure 3.8: Solidworks design of lab on chip (LOC)

After Solidworks design, the mould has been cut using electronic cutter on a sticker paper. Then, the sticker paper that has been cut was placed onto a petri dish to be used as mould in order to develop channel. After that, the PDMS material that consist of Silicon elastomer base and curing agent has been mixed in ratio of 10:1. Basically, to fabricate a layer of PDMS that has 1.5mm thickness, only 10g of Silicon elastomer base and 1g Silicon elastomer curing agent were needed.

The mixture were then stirred and poured onto the petri dish. The petri dish was then placed inside the vacuum chamber for 30 minutes in order to suck out trapped air bubbles. After 30 minutes, the petri dish is let to be dry in room temperature for one day. Then, the dry PDMS was repealed from the petri dish using Isopropanol solution and cut into dimension of 50mmx20mm using cutter. After that, inlets and outlet of the mould were punched using puncher.

Then, the process was repeated two times in order to make the second and third layer that is use to make interface with SPE. Figure 3.9 shows fabrication steps of LOC.

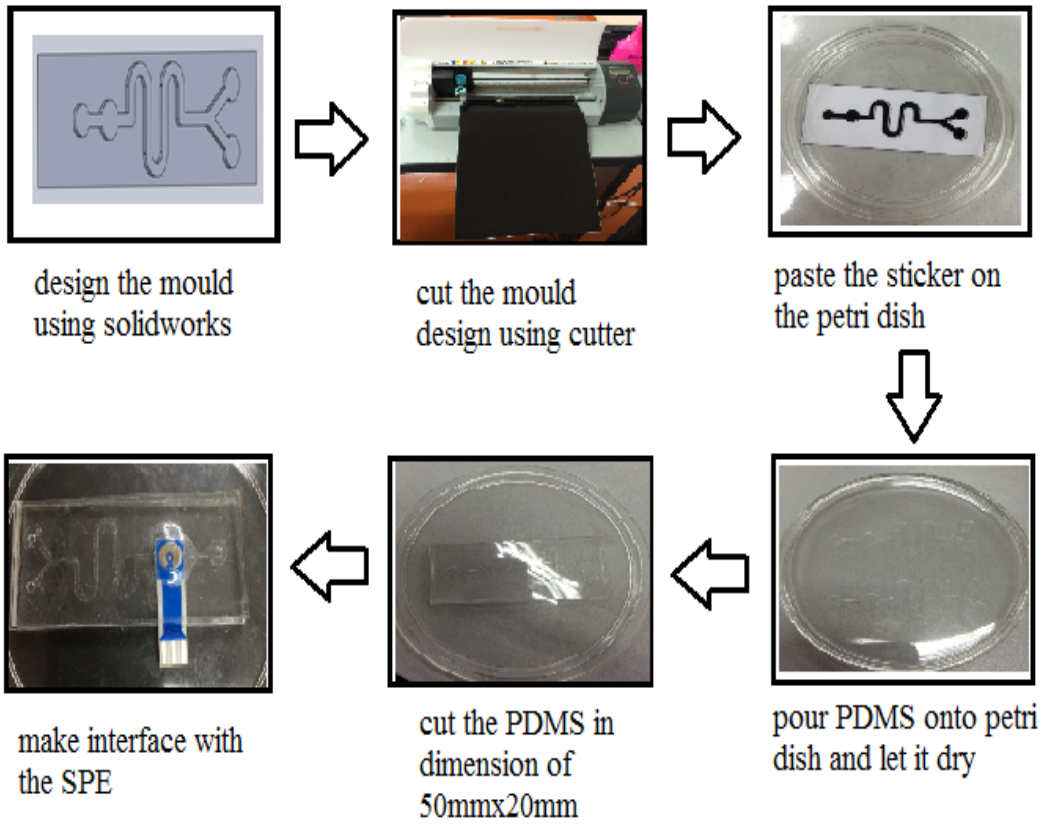
design the mould using solidworks
cut the mould design using cutter
paste the sticker on the petri dish
pour PDMS onto petri dish and let it dry
cut the PDMS in dimension of 50mmx20mm
make interface with the SPE

Figure 3.9: Fabrication process of LOC

3.4.2 Preparation of Solution

Different concentration of glucose solution were prepared using the phosphate buffer solution (PBS) and anhydrous glucose powder. The available high concentration of 1.0M of phosphate buffer solution were diluted using deionized water to 0.1M using ratio 1:10. Then, the glucose stock solution were prepared by mixing 700mg of anhydrous glucose powder into 1L of 0.1M phosphate buffer solution which then produce 1.0M glucose stock solution. Since in this project, the standard glucose level in human body are being referred, therefore, samples of 4mmol, 5mmol, 6mmol and 8mmol glucose concentration were prepared. To prepare the more diluted glucose concentration from 1.0M glucose stock solution, the 0.1M of phosphate buffer solution were added into 1.0M glucose stock solution according to the Equation 3.1 below :

$$M_1V_1 = M_2V_2 \tag{3.1}$$

Basically,M_1 refers to molarity of glucose stock solution, V_1 refers to the volume of glucose stock concentration while, M_2 refers to the desired dilute glucose concentration and V_2 refers to the volume of 0.1M phosphate buffer solution that is need to be added in order to get the desired dilute molarity of glucose concentration.

Another sample which is glucose oxidase solution were prepared by mixing 5mg of the enzyme powder with 200uL, 0.1M phosphate buffer solution. Both of the samples were kept in temperature below -10^0C to ensure the stability of the solution.

3.4.3 Arduino Programming

In this project, two peristaltic pumps were used in order to pump in two different samples inside lab on chip. Therefore, to control the speed of the peristaltic pump, Arduino Uno microcontroller as well as motor driver were used. The motor driver that been used is the shield type that can be placed on top of the Arduino Uno. The Arduino software were used to program the Arduino Uno microcontroller thus to drive the motor driver to control the speed of the peristaltic pumps. Figure 3.10 shows the process flowchart of programming in arduino software.

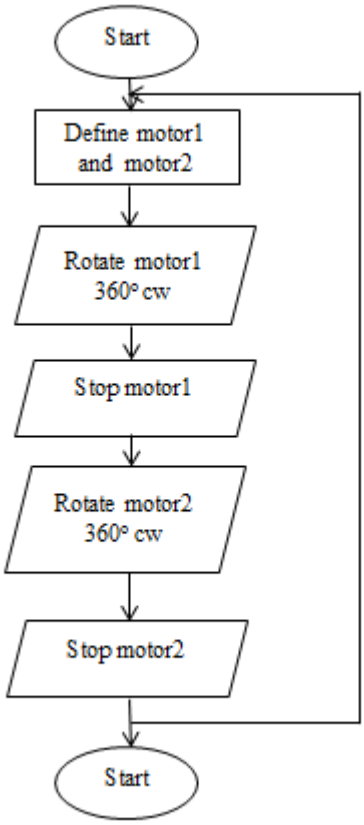

Figure 3.10: Flowchart of programming in Arduino software

In the Arduino software programming, two motors that is motor1 and motor2 were defined. When the switch is turned on, motor1 will start to rotate 360^oclockwise. After motor1 complete a 360^o rotation, it will stop and motor2 will take place and rotate 360^o clockwise. When, motor2 complete a 360^o rotation, it will stop, and the process will be repeated until the switch is turned off.

3.4.4 Emstat And PSTrace Program

Emstat comes together with the PSTrace program that need to be installed through its driver. The Emstat USB cable that has three crocodile clips were then connected to the three electrode of screen printed electrode (SPE) that has been integrated with the lab on chip. Figure 3.11 shows the implementation of Emstat and PSTrace program.

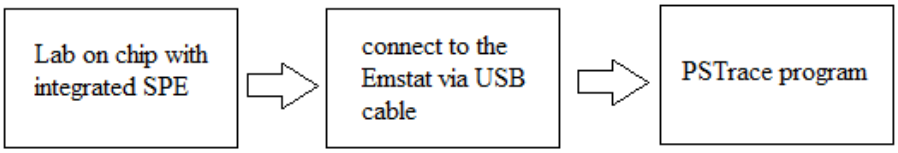

Figure 3.11: Implementation of Emstat and PSTrace program

When the samples of glucose oxidase and glucose solution were pumped into lab on chip, the screen printed electrode will detect the rate of reaction between the samples. Meanwhile Emstat will measure the reaction by cyclic voltametry and amperometric detection techniques. The different measurement technique can be set in the PSTrace program. The PSTrace program will produce cyclic voltametry and amperometric graph based on the reaction between the samples.

Figure 3.12 shows the cyclic voltametry graph of one sample of glucose concentration in PSTrace program.

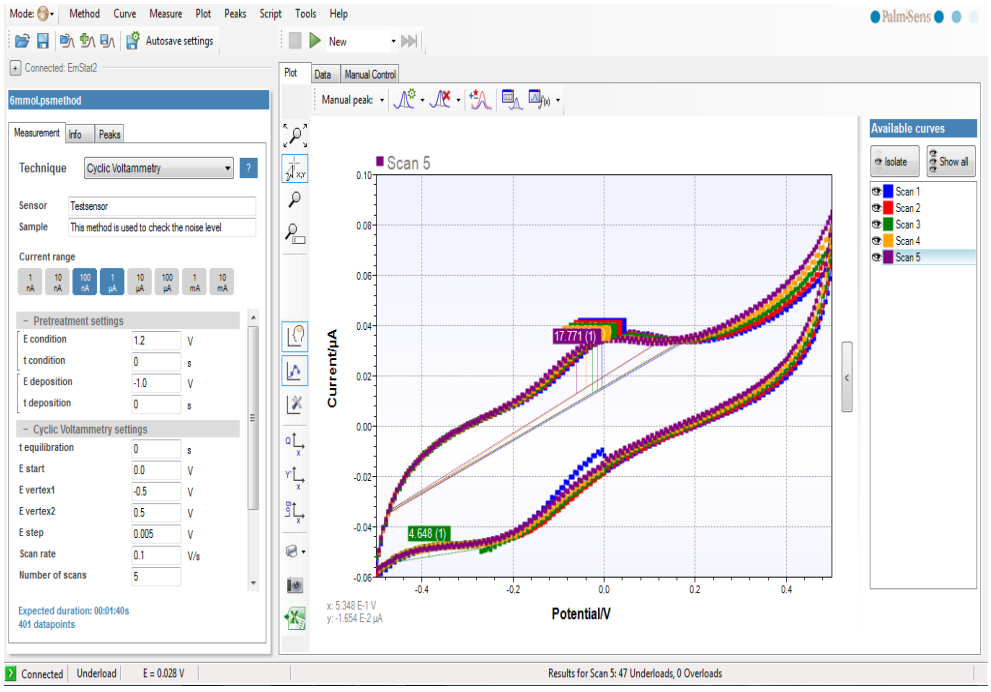

Figure 3.12: PSTrace program using cyclic voltametry measurement technique

By using cyclic voltametry techniques, the voltage range has been set from -0.5V until 0.5V with 100mV/s scan rate which give complete cycle graph.

Figure 3.13 shows amperometric detection graph based on one concentration of glucose solution in PSTrace program.

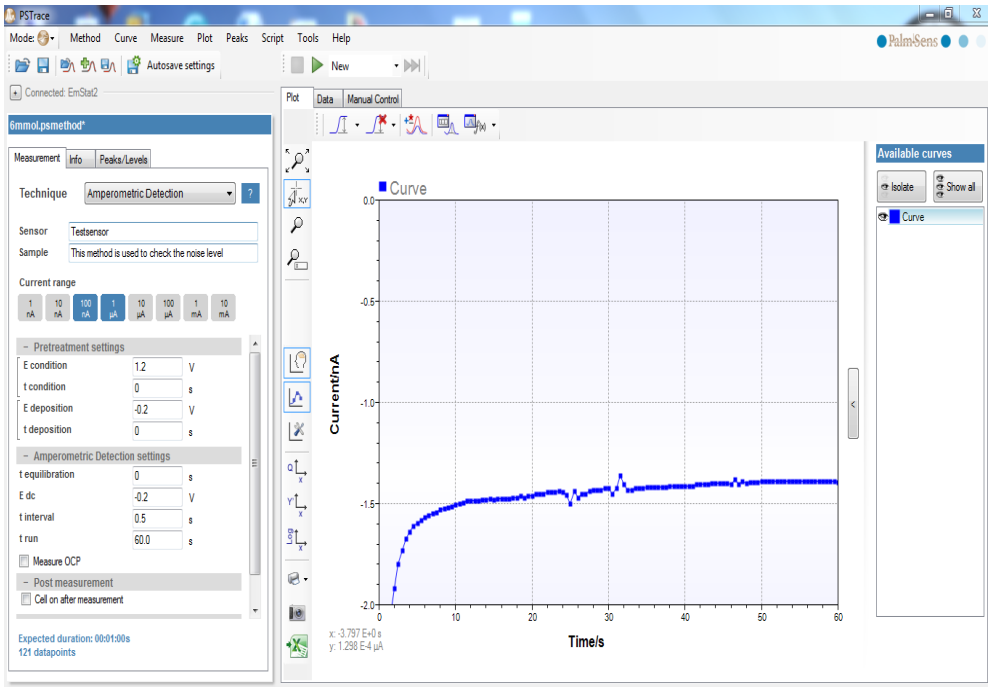

Figure 3.13: PSTrace program using amperometric detection measurement technique

By using amperometric detection technique, the voltage bias is set to -0.2V with 60 second running time.

3.5 Project Management

Project management is a project development that help to achieve the of the objectives of the projects. Project management include the gantt chart for the project planning and cost estimation for the whole system.

3.5.1 Gantt Chart

The Gantt chart in Table 3.3 and Table 3.4 shows the overview of the project implementation during Final Year Project 1 (FYP1) and Final Year Project 2 (FYP2).

Table 3.3: Gantt chart FYP1

WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Understand Topic	■	■	■													
Literature Review			■	■	■	■	■	■	■	■	■	■	■	■		
Select Suitable Glucose sensor			■	■	■	■	■	■								
Design Thinking							■	■								
Present and Submit a proposal									■							
Order Component										■	■					
Learn Solidworks										■	■	■				
Design Lab on Chip											■	■	■	■		
Seminar and Report															■	■

Table 3.4: Gantt chart FYP2

WEEK	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Fabricate Lab On Chip	■	■	■	■												
Integrate SPE with Lab on Chip			■	■	■	■										
Prepare Glucose and Enzyme Solution							■	■	■							
Assemble Hardware							■	■	■	■	■					
Experiment										■	■	■	■	■		
Analyse Result										■	■	■	■	■	■	
Exhibition Preparation															■	■

Basically, FYP1 involves more on understanding the topic by doing research and literature review based on the journals. It also includes the design thinking of the whole CGM system and overall project flow. While, FYP2 involve more on fabrication and hardware implementation in order to get the output data to be analyzed by the PSTrace program.

3.5.2 Cost Estimation

Table 3.5 shows the cost estimation for lab on chip fabrication.

Table 3.5: Cost estimation for lab on chip fabrication

LIST	QUANTITY	UNIT PRICE (RM)	AMOUNT(RM)
1.1 kg Silicon elastomer (base + curing agent)	1	120.96	120.96
Petri dish	3	2.50	7.50
Sticker paper	1	1.20	1.20
Silhouette electronic cutter	1	269.99	269.99
TOTAL			**399.65**

Table 3.6 shows the cost estimation to prepare the glucose and glucose oxidase solution.

Table 3.6: Cost estimation for solution preparation

LIST	QUANTITY	UNIT PRICE (RM)	AMOUNT (RM)
D(+)- Glucose Anhyrdous	1	93.00	93.00
Glucose Oxidase From Aspergillus Niger	1	362.00	362.00
TOTAL			**455.00**

Table 3.7 shows the cost estimation for hardware implementation to run the peristaltic pump and to detect the glucose level.

Table 3.7: Cost estimation for hardware implementation

LIST	QUANTITY	UNIT PRICE	AMOUNT (RM)
Arduino Uno Board	1	97.52	97.52
2A Shield Motor Driver	1	44.00	44.00
Screen Printed Electrode	1	90.00	90.00
Bottle	3	2.60	7.80
Peristaltic Pump	2	43.00	86.00
Silicon tube	1	10.18	10.18
TOTAL			**335.50**

Table 3.8 shows the cost estimation for the whole CGM system where, the total cost for the CGM system is RM1190.15.

Table 3.8: Cost estimation for CGM system

PART	SUBTOTAL (RM)
Lab on chip	399.65
Solution preparation	455.00
Hardware Implementation	335.50
TOTAL	**1190.15**

3.6 Conclusion

In short, the continuous glucose monitoring (CGM) system focus on the fabrication of lab on chip, Arduino programming, and glucose level measurement techniques by using Emstat and PSTrace program that has been implemented in the specific time schedule.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter will discuss about the design and functionality of the project. Therefore, it will give more explanation about the applications of the CGM system and the suitability of the lab on chip design with the CGM system. This chapter also include the result obtained from the reactions of different concentration of glucose solution with the glucose oxidase solution in terms of cyclic voltametry and amperometric detection graph.

4.1 Lab on Chip (LOC)

Using Solidworks 2013, a mould of LOC has been designed. The designed LOC needs to have dimension of 50mm x 20mm with an inlet diameter of 2mm to pump in sample volume of glucose and glucose oxidase. The fluid flow is in spiral shape with 1mm diameter. An outlet with diameter 2mm also included in order to pump out the waste.

The channel of LOC is designed in Y shape inlets that are made to pump in two different samples and spiral shape channel to give more time for the different samples to mix completely in order to get maximum output current. Although it might will be time consuming, but in this project, time is not being a concern since the glucose level needs to be measured continuously through a long period.

The end product of LOC is shown such as in Figure 4.1. The LOC has been successfully fabricated that allows the two different samples of fluids to flow and mix inside the channel without leakage.

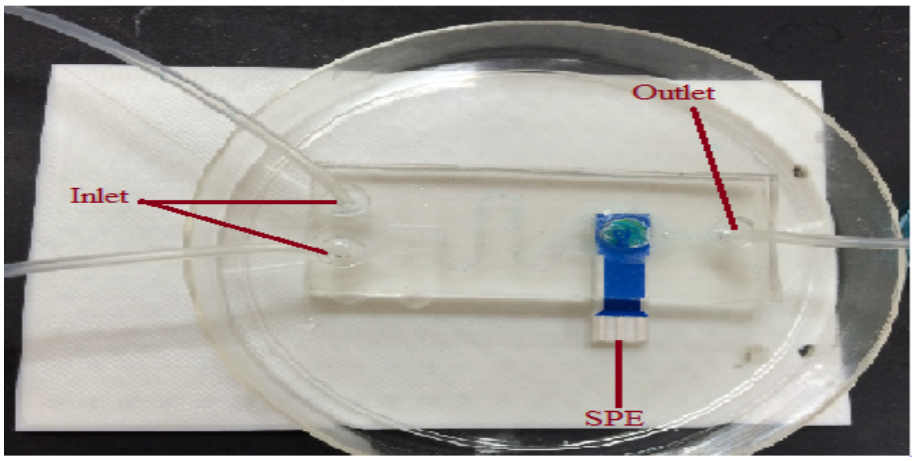

Figure 4.1: Lab on chip

4.2 CGM System

Basically, this CGM system aims to be used during the surgery to monitor the glucose level of a patient continuously. Therefore, the microfluidic device which is lab on chip has been introduced in order to allow the blood of patient to flow through the channel of lab on chip. The lab on chip also has been used as a medium for glucose detection to measure the concentration of glucose by using integrated screen printed electrode.

For this project, the real blood sample is not being used since the focus is more on the design and fabrication of lab on chip. Therefore, the glucose and glucose oxidase has been used as samples to replace the blood. As in Equation 2.1 the glucose level is measured amperometrically by the reaction of glucose and its enzyme which is glucose oxidase in the present of oxygen.

The screen printed electrode has been used to detect the glucose level in this project. Basically, the screen printed electrode has been widely used in the intermittent measurement of glucose such as finger prick. Therefore, by interfacing the screen printed electrode with the lab on chip, the glucose level can be measured continuously through a period of time. For this project, the working electrode and counter electrode of screen printed electrode were made up from gold material , while the reference electrode were made up from the silver material. This type of material were chosen since it provide higher stability towards the glucose reaction compare to other material.

Figure 4.2 shows the hardware implementation of CGM system.

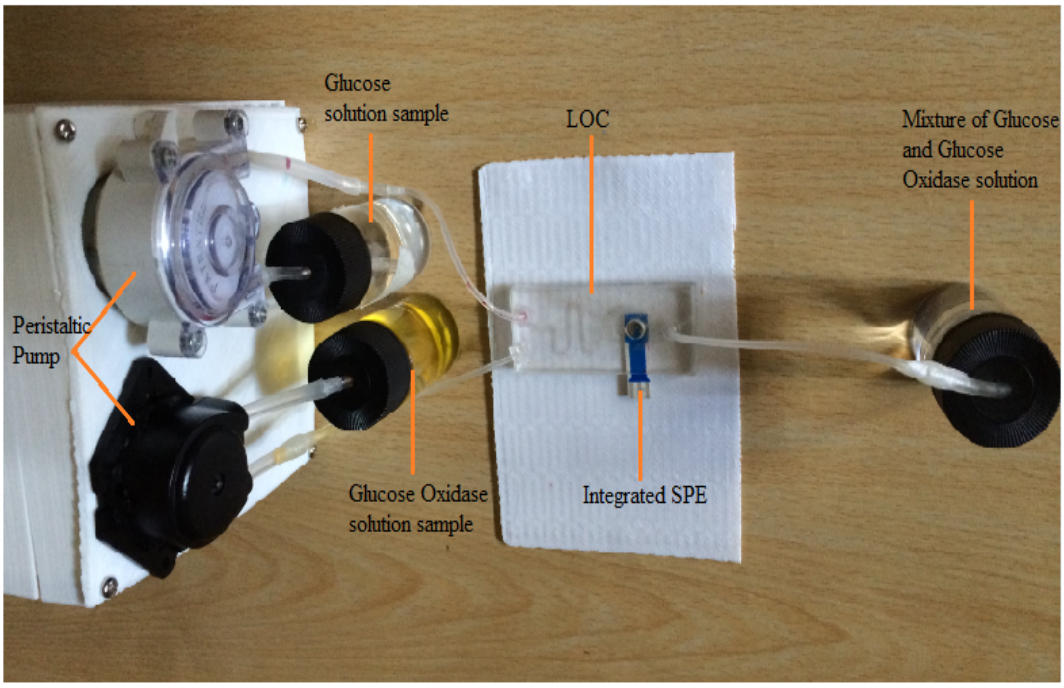

Figure 4.2: Hardware implementation of CGM system

4.3 Data Analysis

Four different concentration samples of glucose solution were used to react with the glucose oxidase solution. The different concentration were prepared according to the standard glucose level in the human body. Normally, the glucose level in human body falls in the range of 70-180mg/dl which is approximately 4mmol/L to 8mmol/L. Therefore, glucose concentration that is 4mmol/L, 5mmol/L, 6mmol/L, and 8mmol/L were prepared as samples.The measurement of each of the concentration were recorded and analyzed. The graph that has been produced in the PSTrace program were exported and combined in Microsoft Excel.

4.3.1 Cyclic Voltametry (CV) Technique

Figure 4.3 shows the cyclic voltametry graph of different concentration of glucose solution. Cyclic voltametry is an electrochemical technique that measure

the current value that has been develop in electrochemical cell. Besides that, it is also being used to study about the mechanism and rates of oxidation and reduction process. Using cyclic voltamery technique, the time dependent potential signal were supplied to the working electrode by Emstat that change its potential in accordance to the fixed potential of reference electrode.While the voltage potential is being supplied, the current flow between the working and counter electrode are being measured which then translated into current against voltage graph.

For glucose and glucose oxidase reaction, to get the complete cycle graph, the voltage is supplied in the range of -0.5V to 0.5V. This range gives complete graph cycle with oxidation and reduction peak. If smaller range are used, it may give distortion to the graph.

As illustrated in the graph, the oxidation and reduction peak of the glucose concentration increases with increasing concentration value.

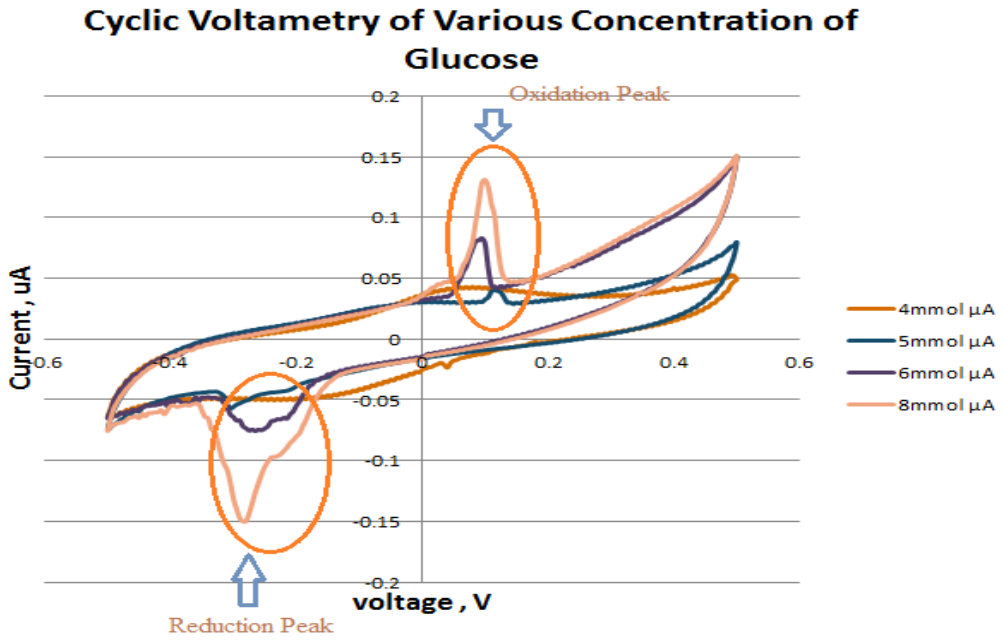

Figure 4.3: Cyclic voltametry graph of various concentration of glucose solution

4.3.2 Amperometric Detection Technique

Amperometry is the changing in electric current due to the detection of ion present in the solution. Therefore, Amperometric detection technique is used to measure the current value based on the detection of ion that present in the glucose with the enzyme.

Figure 4.4 shows the amperometric detection graph based on various concentration of glucose level. The glucose level also shows an increament in the current value with the increasing concentration. The graph shows negative value of current since the amperometric detection measure the reduction reaction of glucose and glucose oxidase.

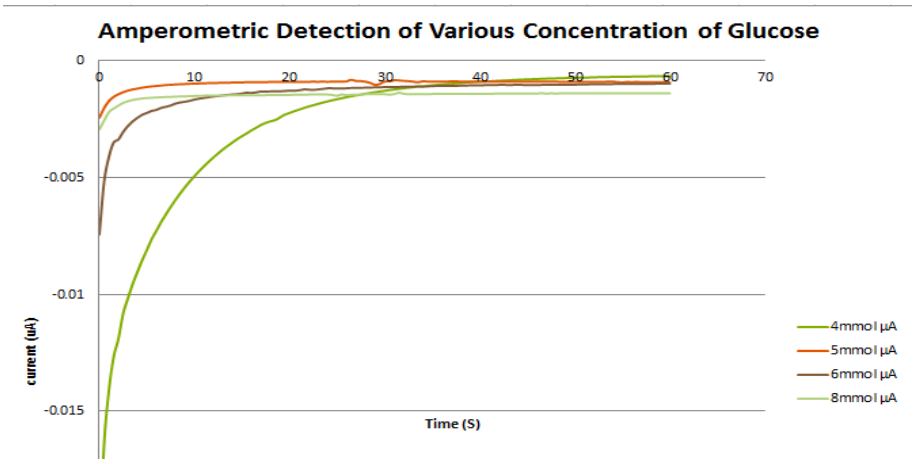

Figure 4.4: Amperometric detection of various glucose concentration graph

Table 4.1 shows the result of current value for four different concentration of glucose solution. Based on the reading, 8mmol/L produce higher current value which is 0.0014μA while the lowest concentration which is 4mmol/L produce lowest current value which is 0.0007μA.

Table 4.1: Result of glucose concentration samples and current reading

Glucose Concentration (mmol/L)	**Current (μA)**
4	-0.0007
5	-0.0009
6	-0.0011
8	-0.0014

4.4 Conclusion

Based on the cyclic voltametry and amperometric detection techniques, it shows that the current values increases as the concentration of glucose increases. Therefore, in normal condition of glucose level, the current value do not fall outside of range 0.0007μA to 0.0014μA. This current measurement condition can be applied during surgery. Therefore, when the current value falls outside this range, the doctor can make preparation to adjust the insulin regimen of the patient blood. Furthermore, the whole CGM system was successfully implemented which has the advantage of smaller size and mobile.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

This chapter give conclusion about the result and the whole CGM process. Recommendation for the future work also included in this chapter.

5.1 Conclusion

A CGM system has been designed so that it can measure the glucose level continuously during the surgery. The design include the whole CGM system as well as the fabrication of Lab on Chip. The Lab on Chip was designed with two inlets and one outlet that has Y shape and spiral channel in Solidworks program. Then, the Lab on Chip was fabricated using PDMS material that consist of silicon elastomer from base and curing agent in ratio of 10:1. The end product of lab on chip that has been integrated with screen printed electrode shows that, the samples were able to flow and mix through the channel without leakage. In addition, the screen printed electrode is able to detect the amperometric reaction between both samples.

Based on the result, the current depicts an increment with the increasing concentration of glucose which is as expected since high concentration of glucose produce more hydogen peroxide and more electron as stated in Equation 2.1.

The whole CGM process also can be applied in the surgery. The peristaltic pump can pump out blood from the blood vessel into the lab on chip which then, the integrated screen printed electrode inside the lab on chip will detect the glucose concentration of blood amperometrically. The blood will then flow back into the blood vessel of the patient body through outlet of lab on chip.

5.2 Limitation

The whole CGM system and process can be applied during the surgery. However, in this experiment, the real blood is not being tested. Instead, the glucose and glucose oxidase has been used as samples to replace the blood. The result may differ and more accurate if using the real blood sample.

5.3 Recommendation

The CGM system is able to measure the glucose level continuously but in invasive way which need to damage the blood vessel. Therefore, for future recommendation, instead of measuring the glucose level invasively, make a smaller lab on chip that can measure the glucose level non-invasively and can be used not only during the surgery, but also in the normal condition.

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APPENDIX A

ARDUINO UNO SOURCE CODE

CODE FLOW TO CONTROL THE SPEED OF PERISTALTIC PUMPS

```
#include "Shield2AMotor.h"


// Connect 4 pin on SHIELD-2AMOTOR to "Signed Magnitude" mode

Shield2AMotor motor(SIGNED_MAGNITUDE);


signed int leftSpeed, rightSpeed;


int i;


void setup()

{
```

```
}


void loop()
{
  // Rotate 360 degree CW
  for(i = 0; i < 50; i++)
  {
    motor.control(100, 0);  //rotate motor1
    delay(10);
    motor.control(100, 0);  //rotate motor1
    motor.control(0, 100);  //stop motor1
    delay(10);
    motor.control(0, 100);  //stop motor1
    delay(10);
    motor.control(0, 100);  //stop motor1
    motor.control(-100, 0);  //rotate motor1
    delay(10);
    motor.control(-100, 0);  //rotate motor1
```

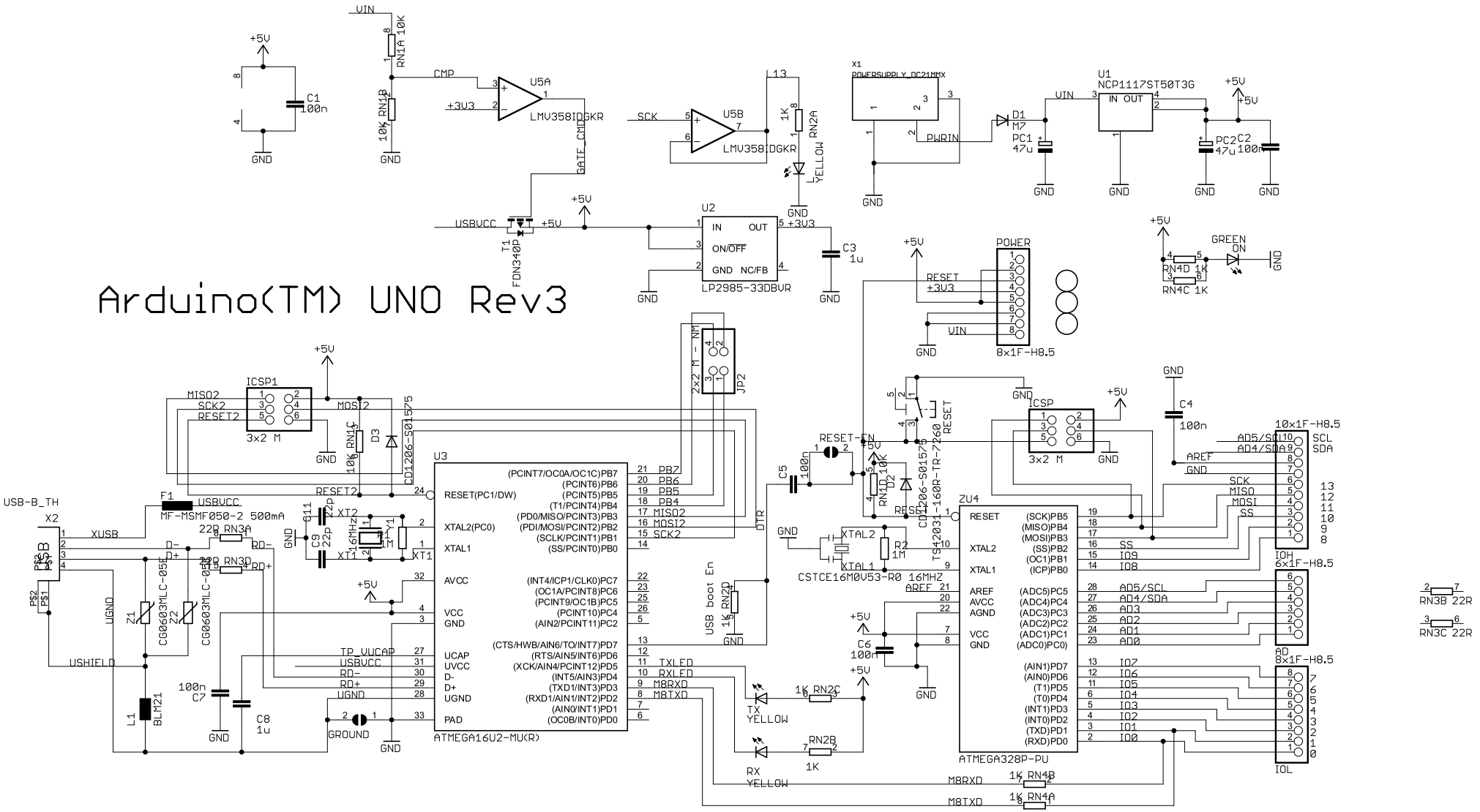
```
  delay(10);
  motor.control(-100, 0);  //rotate motor1
  motor.control(0, -100);  //stop motor1
  delay(10);
  motor.control(0, -100);  //stop motor1
  delay(10);
  motor.control(0, -100);  //stop motor1
  motor.control(100, 0);  //rotate motor1
  delay(10);
}
delay(1000);


// Rotate 360 degree CCW
for(i = 0; i < 50; i++)
{
```

APPENDIX B

DATA SHEET

1. Schematic diagram of Arduino Uno
2. 2A Motor Driver Shield



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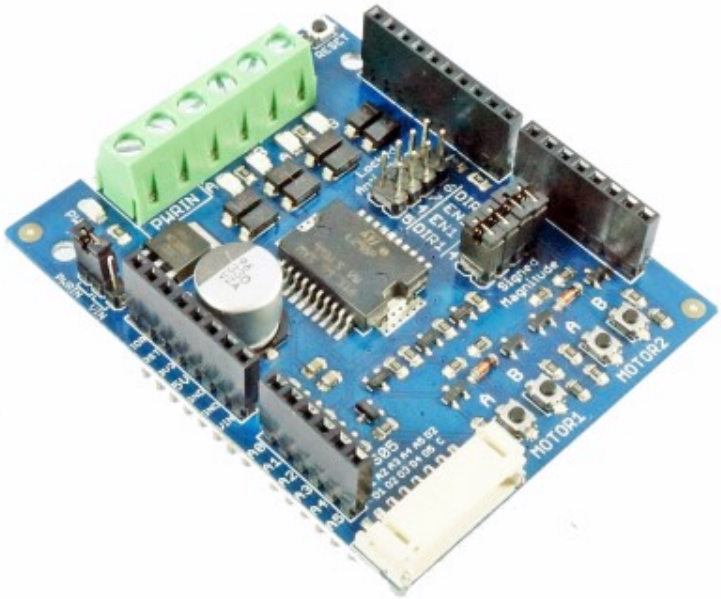
SHIELD-2AMOTOR
Cytron 2A Motor Driver Shield



User's Manual

V1.1

Feb 2015

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1. INTRODUCTION

SHIELD-2AMOTOR is an Arduino shield for controlling dual DC motor up to 2A for each channel. It is compatible with Arduino Uno, Arduino Leonardo, Arduino Mega, Arduino Due and possibly other pin compatible main boards. SHIELD-2AMOTOR uses L298P SMD IC and support for both signed magnitude and locked anti-phase. SHIELD-2AMOTOR shield has stackable side headers which allows for more Arduino shields to be stacked on top of it.

SHIELD-2AMOTOR come with this features:

- Bi-directional control for 2 brushed DC motor.
- Support motor voltage ranges from 5V to 26V.
- Maximum current up to 2A continuous per channel.
- 3.3V and 5V logic level input.
- Stackable I/O header pin.
- Selectable pins for Signed Magnitude and Locked Anti-Phase.
- Test switch for both channel.
- External voltage polarity protector.

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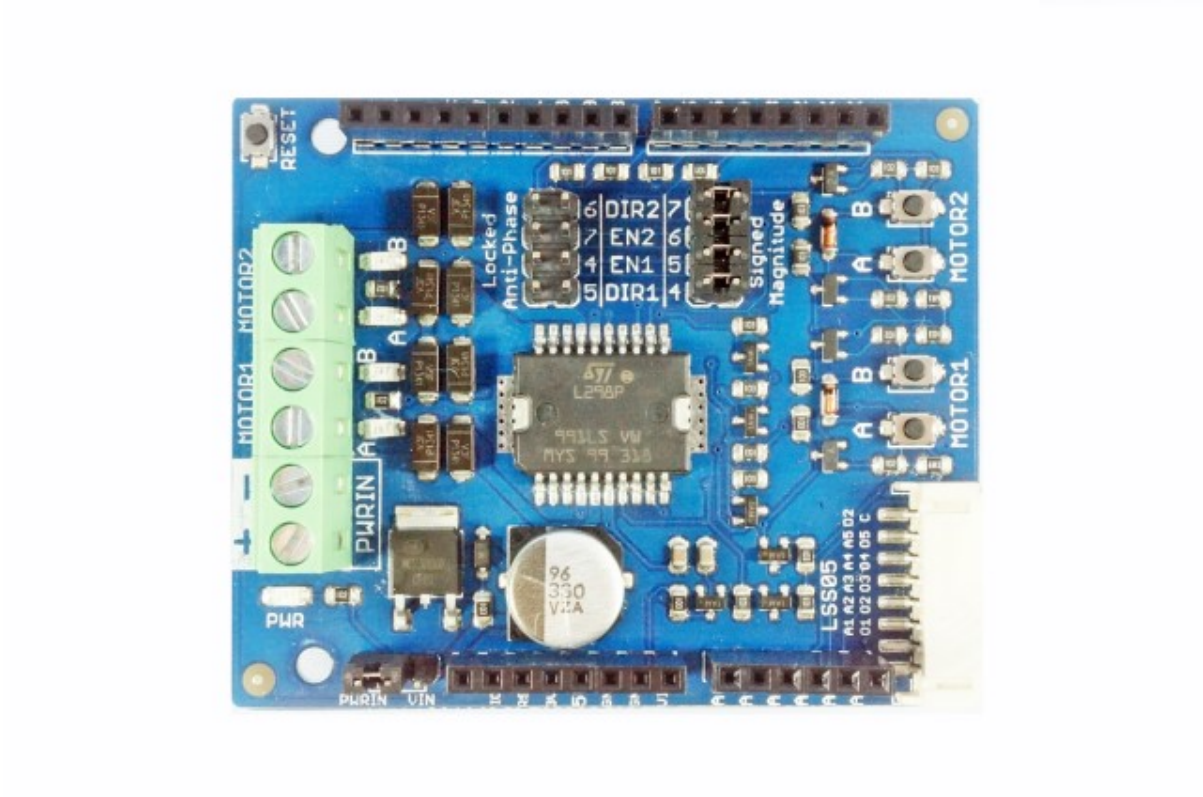
2. PACKING LIST

Please check the parts and components according to the packing list. If there are any parts missing, please contact us at sales@cytron.com.my immediately.



1. 1 x SHIELD-2AMOTOR
2. 5 x mini jumper

3. PRODUCT SPECIFICATION AND LIMITATIONS

Absolute Maximum Rating

Parameter	Min	Typical	Max	Unit
Power Input Voltage (Motor supply voltage)	5	-	26	V
I_{MAX} (Maximum Continuous Motor Current)	-	-	2	A
I_{PEAK} (Peak Motor Current)	-	-	2	A
V_{IOH} (Logic Input-High Level)	3.3	-	5.5	V
V_{IOL} (Logic Input - Low Level)	0	0	0.5	V
Maximum PWM Frequency	-	-	10	KHz

Must not exceed 10 seconds

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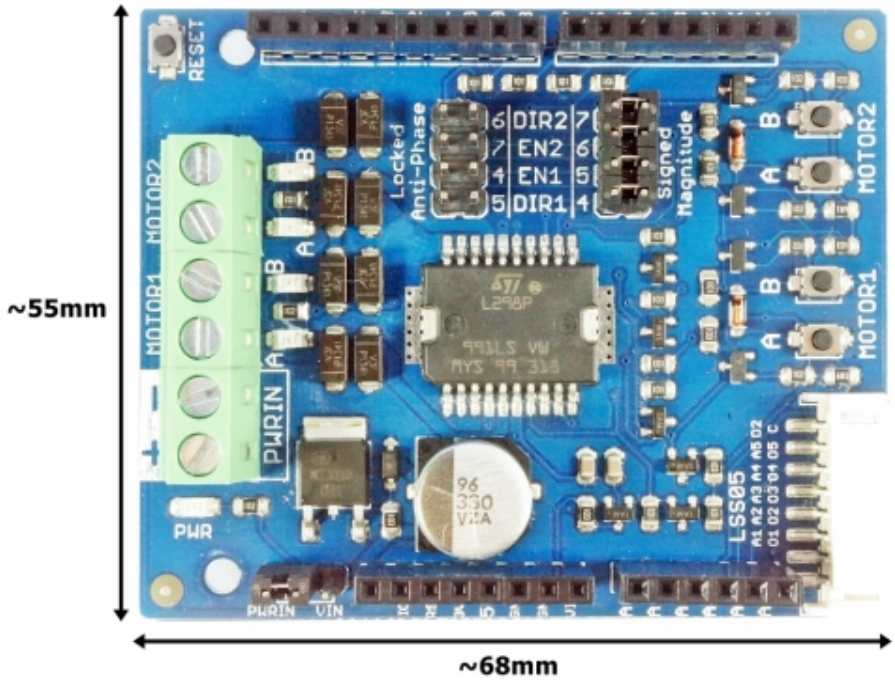
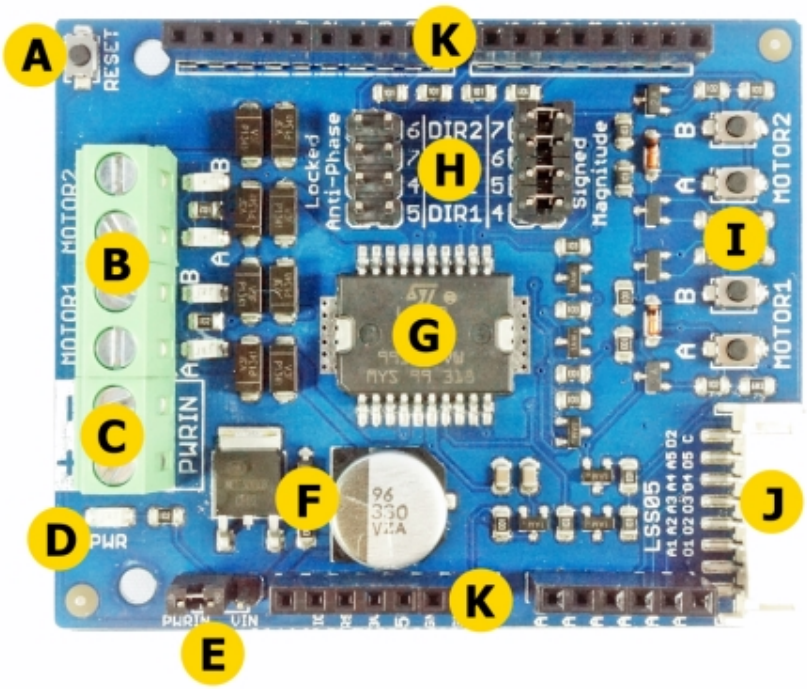
4. DIMENSION


~55mm
~68mm
RESET
MOTOR2
MOTOR1
PWRIN
PWR
VIN
Locked Anti-Phase
Signed Magnitude
6 DIR2 7
7 EN2 6
4 EN1 5
5 DIR1 4
L298P
LSS05

5. BOARD LAYOUT



Label	Function
A	Reset button for Arduino main board
B	Motor connectors.
C	External power (PWRIN) connector.
D	PWR LED.
E	Power source selection.
F	External power (PWRIN) reverse polarity protection circuit.
G	L298P driver motor IC.
H	Motor control mode selection.
I	Test switch.
J	LSS05 connector.
K	Arduino pinout.

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Reset button
User can press this button to restart Arduino program.

Motor connectors
Connect DC motor here.

External power (PWRIN) connector
Connect external power source here.

PWR LED
Motor power indicator.

Power source selection.
User can choose power source either from external (PWRIN) or internal (VIN).

External power (PWRIN) reverse polarity protection circuit.
In case user wrongly connect the external power source polarity, this circuit will protect the shield from broken.

L298P driver motor IC.
SHIELD-2AMOTOR uses L298P driver motor IC.

Motor control mode selection.
User can choose motor control mode either **Signed Magnitude** or **Locked Anti-Phase**.

Test switch.
When button A is pressed, current flows from output A to B and motor will turn CW (or CCW depending on the connection).
When button B is pressed, current flows from output B to A and motor will turn CCW (or CW depending on the connection).

LSS05 connector.
LSS05 can connect directly to this shield and can be interfaced with Arduino.

Arduino pinout.
Other Arduino shield can be stacked on top of this shield.

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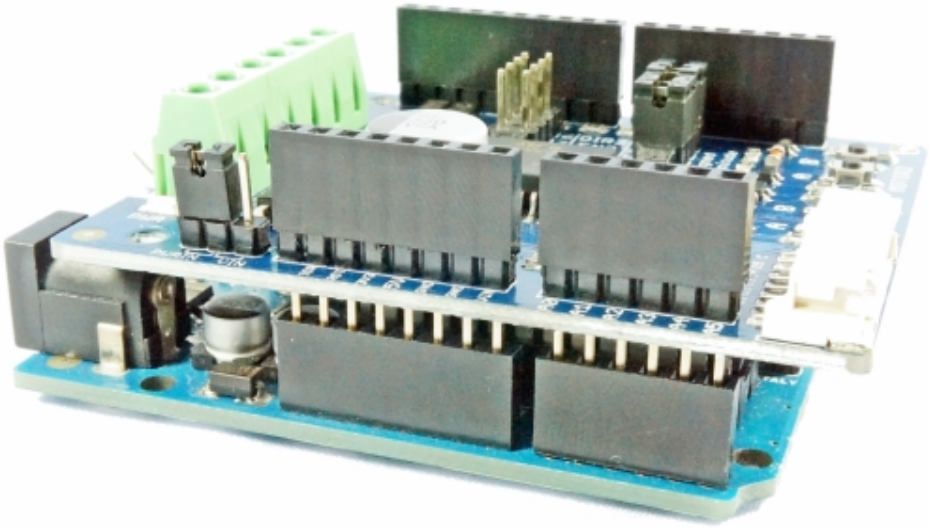
6. HARDWARE

This section shows the example of using SHIELD-2AMOTOR with Arduino Uno as the main controller to control dual brush DC motor. However, other Arduino main board controller can be used (Leonardo, Mega, Due).



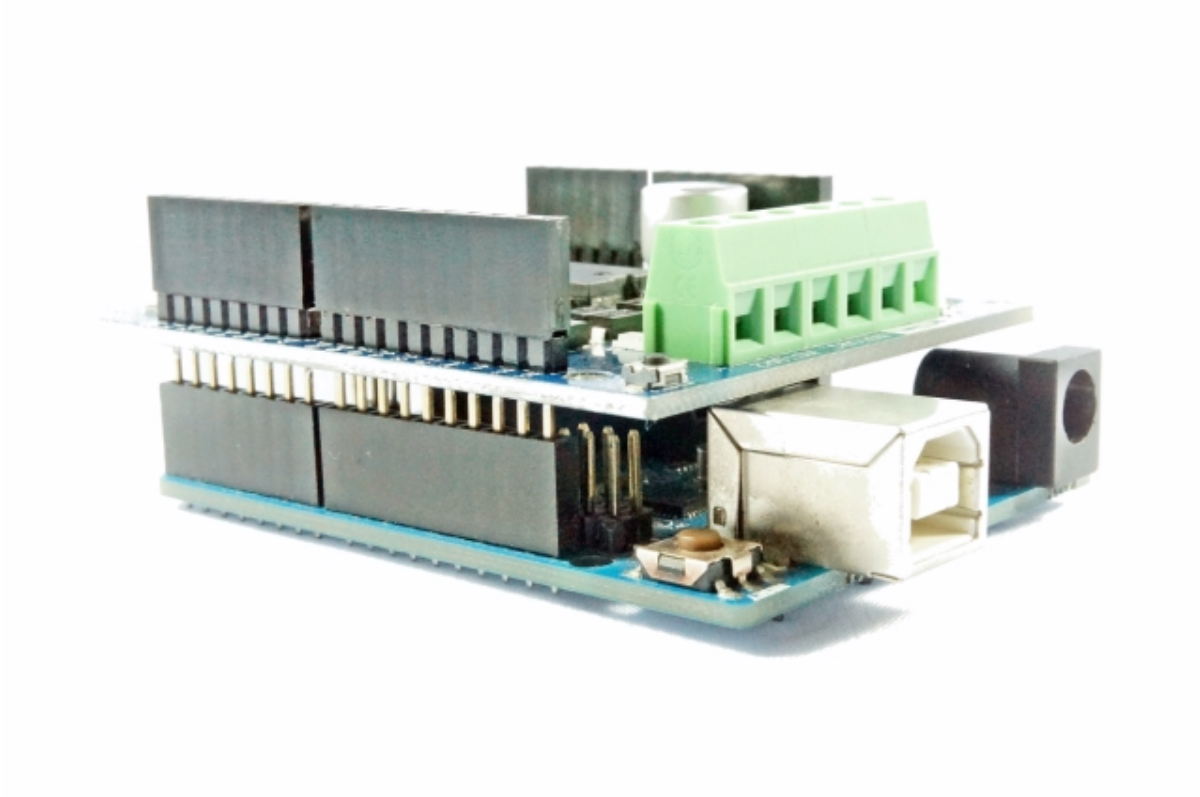
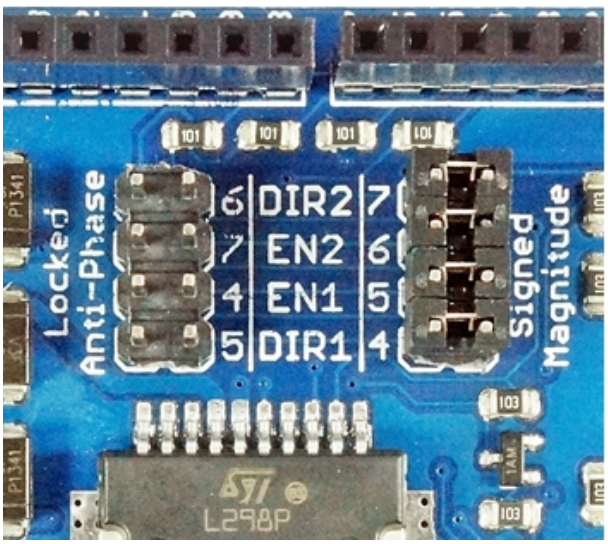
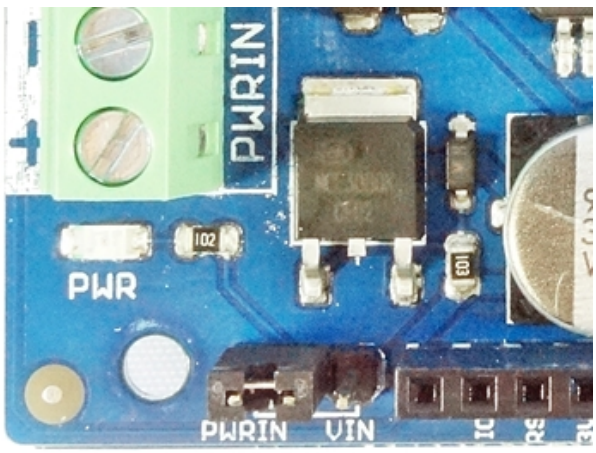


Figure above shows that SHIELD-2AMOTOR is stacked on the Arduino UNO.

Move 4 mini jumpers to Signed Magnitude or Locked Anti-Phase. The default setting is Signed Magnitude. Make sure correct power source is selected (VIN or PWRIN). VIN will connect to the Arduino power source, while PWRIN needs external power source connected to the PWRIN terminal.





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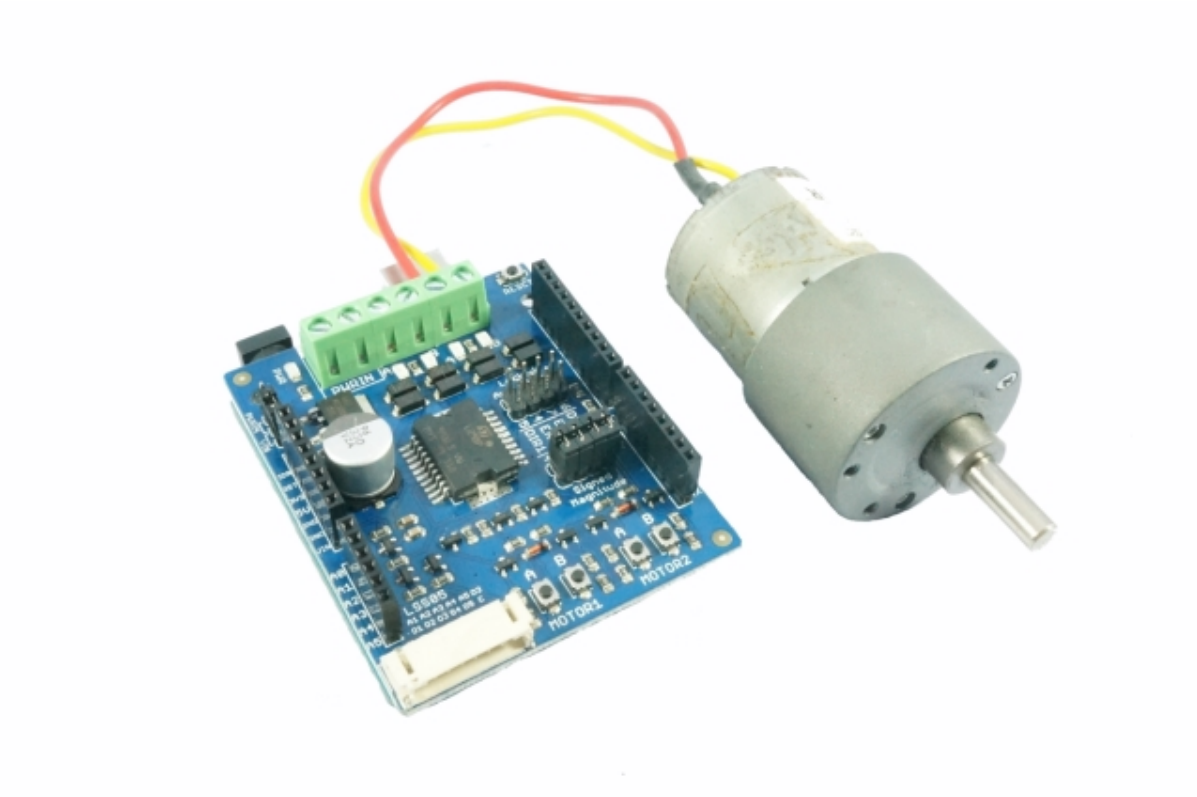
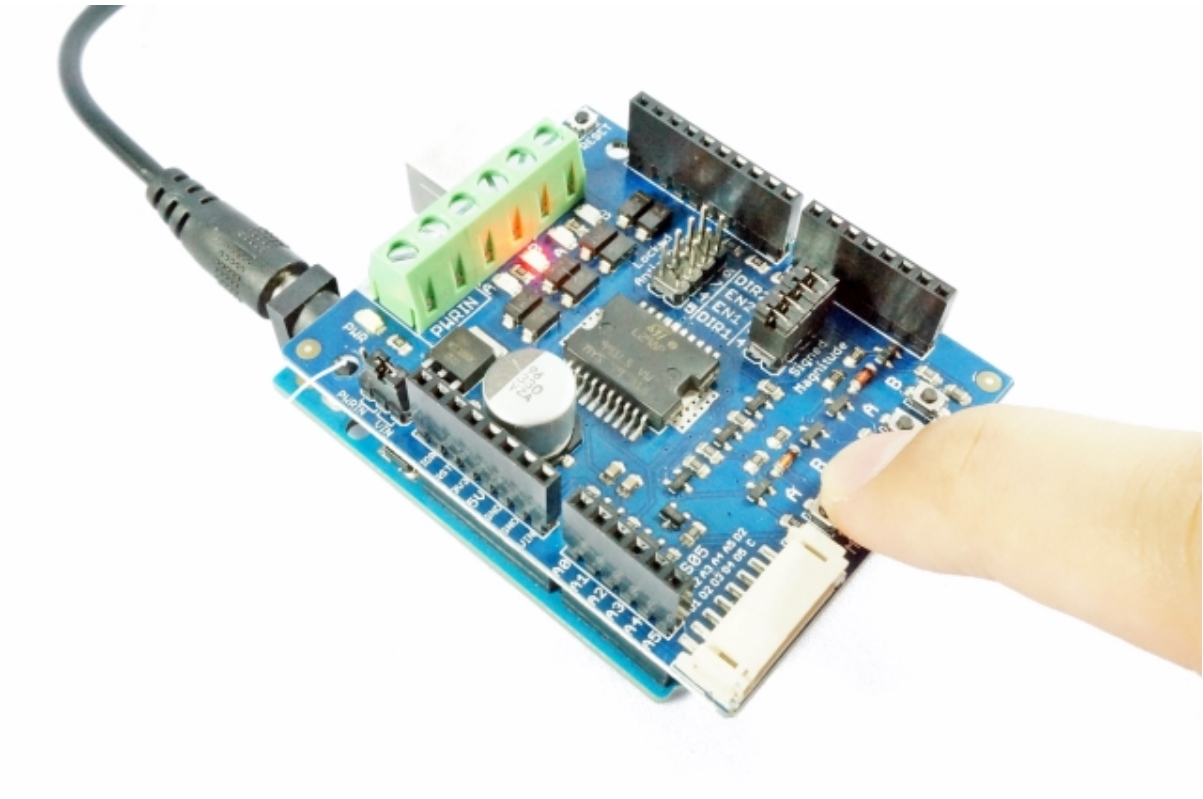


Connect motor to the MOTOR1 and MOTOR2 terminal block. Upload SHIELD-2AMOTOR example code to Arduino. Or you can test directly by using test switch.



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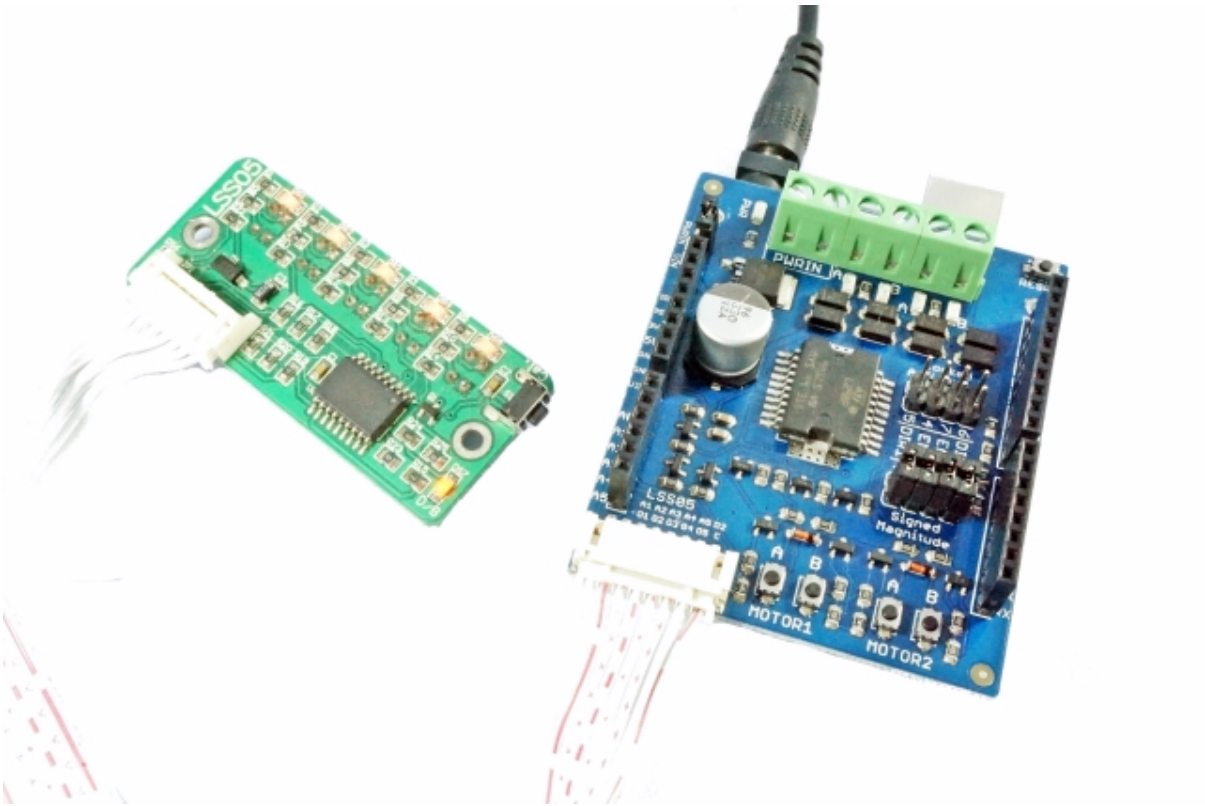
SHIELD-2AMOTOR also include with LSS05 connector. Means you can plug in LSS05 directly to Arduino.



6.1 MOTOR CONTROL PIN

Choose either Locked Anti-Phase or Signed Magnitude mode by moving the 4 mini jumpers on shield. Below is a table to summarize pin used for motor control.

	Locked Anti-Phase (Arduino Pin)	**Signed Magnitude (Arduino Pin)**
DIR 2	6	7
EN 2	7	6
EN 1	4	5
DIR 1	5	4

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6.2 LSS05 PIN

LSS05 can connect directly to this shield and can be interfaced with Arduino. Below is a table showing the pins connection between LSS05 and Arduino on 2A motor driver shield.

Arduino Pin	LSS05 Output Pin
5V	5V
GND	GND
A1	01
A2	02
A3	03
A4	04
A5	05
D8	Cal

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7. SOFTWARE

Arduino library and example code can be downloaded from the SHIELD-2AMOTOR product page at Cytron's website.

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8. WARRANTY

- Product warranty is valid for 12 months.
- Warranty only applies to manufacturing defect.
- Damaged caused by misuse is not covered under warranty
- Warranty does not cover freight cost for both ways.

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